Research Article

Integrated Therapy of Diet and Ayurvedic Drug may be a Safer Choice in the Prevention of Oxidative Stress-induced Diabetes Mellitus

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ABSTRACT

Herbal drug therapy and restricted diet (RD) are known to improve diabetes mellitus (DM) and its associated problems. Here, we aimed to examine the importance of RD alone or in combination with a polyherbal drug, Diabecon in the prevention of alloxan induced DM. For this young diabetic rats were either kept on 50% RD and/or treated with Diabecon (2 gm /Kg body weight) for two months and then different indices were estimated. Finding revealed a significant increase in serum glucose, G6Pase, atherogenic index (AI), tissue oxidative stress with decrease in insulin and hepatic glycogen content in diabetic rats. While rats kept on either therapy exhibited significant improvement in most of the parameters, best protective effects were observed in animals which received the drug and were also under RD therapy. In conclusion, RD and polyherbal drug therapy cured the remedy and exhibited additive protection against oxidative stress.

Keywords: Diet restriction, combined therapy, diabetes, glucose-6-phosphatase, oxidative stress.

INTRODUCTION

Both, type 1 and type 2 diabetes mellitus (DM) are considered as heterogeneous metabolic syndromes that affect multiple biochemical processes[1,2]. Unfortunately, the incidences of DM and overweight are also going up every year, which are prominently linked with irregular diet style[2-4]. In addition, onset of DM further worsens the normal functioning of liver, kidney, heart and almost all other body organs[4]. Recent data showed that the tendency of fast food consumption, heavy diet, sedentary life style and oxidative environment are emerging out as responsible factors for enhancing epidemiology of metabolic syndromes in developed and developing countries[5-7].

Though reactive oxygen species (ROS) play central role in most physiological processes, their overproduction causes damage of lipids, proteins and nucleic acids.[6,7] and also impair the normal defending capabilities of tissues[3,7,8]. These abnormalities further create more oxidative environment and worsen diabetic complications[8-10].

Several categories of conventional, traditional and a number of other newly designed drug therapies have been launched to regulate DM, although not highly effective[11,12].

However, restricted diet (RD) has been found to increase the expression of genes involved in antioxidant defence, nutrient sensing, insulin sensitivity, β-cell functions, carbohydrates and fat metabolism etc[5,13]; and hence may benefit diabetics health and reduce complications of DM[13,14]. On the other hand, phytochemicals based remedies are also considered effective, economic and safe medication that work in parallel approach[15-17]. Moreover, polyherbal drug therapy is found to be superior than that of individual one[17-19], so here, the comparative as well as combined effects of RD and polyherbal formulation on young diabetic rats was evaluated. The possible additional effects of mentioned parameters may help to diminish or delay the onset of DM. As, liver and kidney are primarily involved in drug metabolism and detoxification, they were tested for drug/diet induced tissue toxicity[4,20-21] and because the chronic diabetes may result in cardiac problems[3,16], same parameters were also tested in this tissue.

To the best of our knowledge this is the first study which reveals the cumulative as well as individual effects of long term herbal drug treatment with restricted diet in diabetic rat model.

MATERIALS AND METHODS

Chemicals

While 5,5,dithiobis-2-nitrobenzoic acid (DTNB), glucose 6-phosphate, Ellman's reagent, alloxan monohydrate and Carboxy methyl cellulose (CMC), 2,4-dinitropheno hydrazine (DNPH), xylenol orange and ascorbic acid were purchased from Hi-media, Mumbai, India; diethylene triamine penta acetic acid (DTPA), Tris-(hydroxymethyl)-amonium methane, sodiumdodecyl sulphate (SDS), thiobarbituric acid (TBA), ethyelene-diaminetetra-acetic acid (EDTA), pyrogallol, hydrogen peroxide (H_2O_2) were obtained from E. Merck Ltd. (Mumbai, India). All other chemicals were of reagent grade and obtained from Loba chemie (Mumbai). Diabecon was purchased from registered local market store. Radioimmunoassay (RIA) kit for estimation of total serum insulin was purchased from Beckman Coulter, New Delhi, India.

Drug Preparation

Diabecon (Himalaya Drug Company, Bangalorea, Batch no. 37200283B) is a polyherbal formulation of about forty two well-known antidiabetic herbs, extracts and bhasma.

Aqueous suspension of Diabecon was prepared in 0.5% CMC, a suspending agent[22] and drug was administered at constant volume 0.1 ml/ rat p.o., for two months.

Animals

Healthy colony bred Wistar male rats of 118±12 g (aged 4-5 weeks) were housed in polypropylene cages under constant temperature (27±1°C) and photo schedule (14 h light+ 10 h dark). They were provided standard rat feed (Golden feeds, New Delhi, India) *ad libitum* and had free access to drinking water. Standard ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environmental and forests, Govt. of India were followed after the approval of Departmental Ethical Committee (Registration no. 779) for Handling and Maintenance for Experimental Animals.

Experimental Design

Thirty-five rats were divided into five groups of seven each. Group 1 (Control) and Group 2 (diabetic) received only 0.1 ml aqueous suspension of 0.5% CMC (drug solvent) with normal diet. While group 3 (DT) animals were given normal diet with Diabecon at 2 g/kg /day, group 4 (RD), was given 50% diet restriction with 0.1 ml 0.5% CMC without any drug, animals of group 5 (DT+RD) received 50% RD + Diabecon at 2 g/kg/day for consecutive 60 days. On 0th day 16 hours fasted animals of group 2-5 were given single injection of 100 mg/kg (i.p.) of alloxan monohydrate prepared in normal saline. While those of group 1 received only 0.1 ml normal saline to ascertain the effect of vehicle, if any. Drug was administered at a fixed time (10:00-11:00 AM) of the day to avoid circadian variation. Body weight (BW) and water intake (WI) were also measured.

At the end of the experiment overnight fasted animals were sacrificed by cervical dislocation. Blood was collected from each one (without any coagulant) and serum was separated by centrifugation. Liver, kidney and heart were quickly removed, washed in cold phosphate buffer saline (PBS; 0.1 M, pH 7.4) and homogenized in PBS with the help of a motor driven teflon homogenizer. The homogenates were then centrifuged at 15,000Xg at 4 °C for 30 min to obtain a clear supernatant which was then transferred to the other tubes and stored at -20 °C for further analyses.

Biochemical Analysis

Total circulating serum insulin was estimated by RIA in Medicare hospital, Indore, India following the protocol provided in the kit (Beckman Coulter, India Pvt Lmd).

LPO was determined as TBARS adduct formed by reaction of TBA and malondialdehyde (MDA), measured at 532 nm (Extinction coefficient, E = 1.56 x 10^5), using a Shimadzu UV-1700 spectrophotometer[23]. Lipid hydroperoxide and Protein carbonyl content were estimated by the method of Hicks and Gebicki[24] and Smith[25], respectively. The protein

content was determined by following the protocol of Lowery[26] using bovine serum albumin (BSA) as standard.

Activity of SOD, CAT, GPx and reduced glutathione (GSH) were determined following the protocols as routinely done in our lab[8,16,22,27-29]

Glycogen was measured by the protocol of Carroll[30]. For glucose-6-phosphatase (G-6-Pase) activity the method of Baginski[31] was followed. Serum glucose, different lipid profile parameters, SGOT, SGPT, creatinine and blood urea nitrogen (BUN) were estimated using commercially available kits, purchased from ERBA, Transasia Bio-medicals Ltd., Solan, India, as routinely done in our laboratory[16,32-34].

Statistical Analysis

All values were expressed as mean ± SEM. Differences in mean values were compared using Prism software, version 5.1 for windows, Inc., La Jolla, CA, USA by one way analysis of variance (ANOVA) followed by t-test. P<0.05 was considered as statistically significant.

RESULTS

The serum glucose level was profoundly elevated (288.59% than control group) in alloxan induced diabetic rats, which was significantly decreased in DT (60.22%) and RD (60.03%) animals.

However, maximum reduction in serum glucose (69.92%) was observed in DT+RD rats. A significantly decreased level of serum insulin and hepatic glycogen content was also observed in diabetic rats (*P<0.0001* for both), which were significantly improved in drug treated group. (Table-1).

The increased activity of G6Pase enzyme was also observed to be normalized in rats receiving any of the therapies mentioned above.

Thiobarbituric acid reactive substances (TBARS), hydroperoxides and protein carbonyl levels were significantly increased in studied tissue of diabetic group (*P<0.0001*, *P<0.0001* and *P<0.001* respectively) as compared to their control values. However, chronic treatment with DT or RD reduced the same. Although the animals kept on DT+RD showed a significant reduction in same parameters, the values were lesser than any of the individual treatments. In case of renal and cardiac tissues also rats kept on combined therapy showed not only significant (*P<0.01, P<0.05* and *P<0.001* respectively), but a greater fall in the same than any individual therapy treated animals (Tables 2-4).

Tables 2, 3 & 4 show the activities of SOD and CAT in different groups of rats. The present findings revealed that Diabecon treated rats showed significantly increased activity of the same enzyme in kidney (*P<0.001*) and in heart (*P<0.05*); but non-significant increase was observed in hepatic SOD as compared to diabetic rats. Additive results were found following DT+RD treatments. Diet

restriction alone or along with ayurvedic drug exhibited a significantly increased activity of CAT.

In case of GPx and GSH also, significant improvement was seen in renal tissues of DT+RD animals as compared to the animals kept on individual therapy.

When the changes in body weight (BW) were compared after two months, a significant increase was found in all groups.

Control and diabetic rats showed 33.82% and 17.29% increase in BW respectively; while the % increases in the BW of DT, RD and DT+ RD were found as 28.49%, 20.81% and 33.22% respectively.

In case of water consumption (a mean value of last 06 days), a significant increase in diabetic group (*P<0.01*) was recorded than control group (Table-1).

As depicted in Table 5, a significantly increase abnormal lipid profile was seen in diabetic control group while, in DT, RD and DT+RD treated rats the values of the same were found nearly normal.

In case of HDL, a significant (*P<0.01*) enhancement was found in the rats kept on combined treatment.

The liver function indicating enzymes serum glutamic oxaloacitic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), and renal function indicators such as creatinine and blood urea nitrogen (BUN) also seem to be normalized in animals kept on different therapies.

On the contrary, no significant difference was found in the activity SGPT and SGOT among DT, RD and DT+RD groups (Fig 1 and 2).

Table 1: Effects of Diabecon (2 gm/kg BW/day) or restricted diet (RD; 50% of the control) either alone or both (Drug+RD) for 60 days on fasting serum glucose (FSG, mg/dl), insulin (IU/L), hepatic glycogen (mg glycogen/100 gm tissue), glucose 6-phosphatase (G6-Pase, μM inorganic phosphate liberated/ min/ mg protein), body weight (BW, % increase) and water intake (WI, ml/rat/day) of mice.

Parameters	Control	Diabetic	Diabetic + Drug	Diabetic + RD	Diabetic +Drug+ RD
FSG	93.79 ± 4.78	364.49 ± 19.70^z	144.97 ± 7.06^d	145.68 ± 8.97^d	109.63 ± 5.41^{d,p,m}
Insulin	11.89 ± 0.72	6.73 ± 0.81^z	8.98 ± 0.83^b	8.14 ± 0.76^a	8.58 ± 0.64^b
Glycogen	119.09 ± 5.56	84.02 ± 5.19^z	114.40 ± 6.23^d	121.73 ± 8.90^d	133.95 ± 8.62^{d,q,n}
G6-Pase	1.67 ± 0.12	3.46 ± 0.22^z	2.33 ± 0.17^b	1.92 ± 0.19^c	1.62 ± 0.13^{d,p}
BW	36.65 ± 1.76	19.18 ± 3.05^y	27.32 ± 5.70	21.62 ± 4.38	37.58 ± 2.66^{c,m}
W I	12.00 ± 0.41	14.80 ± 0.52^x	13.33 ± 0.31^a	14.25 ± 0.77	13.25 ± 0.43^a

^{x}P<0.01, ^{y}P< 0.001 & ^{z}P< 0.0001 as compared to respective control; ^{a}P<0.05, ^{b}P< 0.01 ^{c}P< 0.001 & ^{d}P< 0.0001 as compared to respective diabetic group; ^{p}P< 0.01 & ^{q}P< 0.001 as compared to respective drug treated group; ^{n}P< 0.05 & ^{m}P< 0.01 as compared to respective restricted diet treated group.

Table 2: Effects of Diabecon (2gm/kg BW) or restricted diet (RD; 50% of the control) either alone or both (Drug + RD) for 60 days on the alterations in hepatic tissue. LPO (lipid peroxidation; nM MDA formed/ mg protein/ hr), LOOH (lipid hydroperoxide; nM hydroperoxide formed/ mg protein), PC (protein carbonyl; nmol carbonyl/ mg Protein), SOD (superoxide dismutase; U/ mg protein), CAT (catalase; μM H_2O_2 decomposed/ min/ mg protein) and GSH (reduced glutathione; μM reduced glutathione/mg protein)

Parameters	Control	Diabetic	Diabetic + Drug	Diabetic + RD	Diabetic + Drug+ RD
LPO	1.04 ± 0.10	2.35 ± 0.09^z	1.41 ± 0.07^d	1.10 ± 0.05^d	0.75 ± 0.09^{d,q,m}
LOOH	1.62 ± 0.06	3.51 ± 0.25^z	1.995 ± 0.09^d	2.45 ± 0.05^b	1.63 ± 0.06^{d,p,m}
PC	1.33 ± 0.12	3.51 ± 0.47^y	2.09 ± 0.21^a	1.89 ± 0.19^b	1.69 ± 0.14^b
SOD	5.12 ± 0.60	3.38 ± 0.42^x	4.52 ± 0.42	5.85 ± 0.39^b	6.10 ± 0.42^{c,p}
CAT	34.34 ± 2.80	24.34 ± 1.81^y	34.11 ± 2.07^b	39.91 ± 2.74^c	40.64 ± 2.70^c
GSH	5.76 ± 0.31	3.35 ± 0.56^x	5.26 ± 0.67^a	5.71 ± 0.28^b	6.35 ± 0.81^c
GPx	7.98 ± 0.51	5.43 ± 0.43^x	7.60 ± 0.67^b	7.88 ± 0.21^c	8.27 ± 0.81^c

^{x}P<0.01, ^{y}P<0.001 & ^{z}P<0.0001 as compared to respective control; ^{a}P<0.05, ^{b}P<0.01 ^{c}P<0.001 & ^{d}P<0.0001 as compared to respective diabetic group; ^{p}P<0.01 & ^{q}P<0.001 as compared to respective drug treated group; ^{n}P<0.01 & ^{m}P<0.001 as compared to respective restricted diet treated group.

Table 3: Effects of Diabecon (2 gm/kg BW) or restricted diet (RD; 50% of the control) either alone or both (Drug +RD) for 60 days on the alterations in renal tissue. LPO (lipid peroxidation; nM MDA formed/ mg protein/ hr), LOOH (lipid hydroperoxide; nM hydroperoxide formed/ mg protein), PC (protein carbonyl; nmol carbonyl/mg Protein), SOD (superoxide dismutase; U/mg protein), CAT (catalase; μM H_2O_2 decomposed/min/mg protein) and GSH (reduced glutathione; μM reduced glutathione/ mg protein)

Parameters	Control	Diabetic	Diabetic + Drug	Diabetic + RD	Diabetic + Drug + RD
LPO	1.46 ± 0.15	3.74 ± 0.26^y	2.23 ± 0.19^c	2.04 ± 0.19^c	1.57 ± 0.12^{d,p}
LOOH	2.01 ± 0.08	3.66 ± 0.29^y	2.24 ± 0.15^b	2.847 ± 0.11^a	1.68 ± 0.14^{d,p,m}
PC	0.55 ± 0.07	1.34 ± 0.08^z	1.02 ± 0.05^b	0.74 ± 0.05^d	0.69 ± 0.03^{d,q}
SOD	5.19 ± 0.99	3.16 ± 0.23^y	5.02 ± 0.32^c	6.92 ± 0.4^d	5.67 ± 0.47^c
CAT	63.71 ± 3.43	36.92 ± 2.89^z	44.83 ± 1.18^a	52.42 ± 2.11^b	54.67 ± 2.39^{c,p}
GSH	6.88 ± 0.95	2.40 ± 0.23^y	3.94 ± 0.30^b	4.58 ± 0.33^c	5.06 ± 0.34^{d,p}
GPx	8.22 ± 0.33	6.11 ± 0.34^y	7.50 ± 0.28^b	7.77 ± 0.25^b	8.24 ± 0.29^c

[x]P<0.01, [y]P<0.001 & [z]P<0.0001 as compared to respective control; [a]P<0.05, [b]P<0.01 [c]P<0.001 & [d]P<0.0001 as compared to respective diabetic group; [p]P<0.01 & [q]P<0.001 as compared to respective drug treated group; [n]P<0.01 & [m]P<0.001 as compared to respective restricted diet treated group.

Table 4: Effects of Diabecon (2gm/kg BW) or restricted diet (RD; 50% of the control) either alone or both (Drug+RD) for 60 days on the alterations in cardiac tissue. LPO (lipid peroxidation; nM MDA formed/ mg protein/hr), LOOH (lipid hydroperoxide; nM hydroperoxide formed/mg protein), PC (protein carbonyl; nmol carbonyl/mg Protein), SOD (superoxide dismutase; U/mg protein), CAT (catalase; μM H_2O_2 decomposed/min/mg protein) and GSH (reduced glutathione; μM reduced glutathione/ mg protein)

Parameters	Control	Diabetic	Diabetic + Drug	Diabetic + RD	Diabetic +Drug+ RD
LPO	0.51 ± 0.09	2.06 ± 0.32^y	1.53 ± 0.26	1.39 ± 0.13	0.79 ± 0.05^{b,p,n}
LOOH	0.78 ± 0.08	1.84 ± 0.12^z	0.89 ± 0.06^d	0.91 ± 0.08^d	0.75 ± 0.06^d
PC	1.28 ± 0.09	2.69 ± 0.217^z	1.89 ± 0.197^b	1.66 ± 0.14^c	1.156 ± 0.11^{d,p,n}
SOD	4.38 ± 0.52	2.95 ± 0.33^x	4.25 ± 0.48^a	4.83 ± 0.23^c	4.92 ± 0.37^b
CAT	31.17 ± 2.05	21.29 ± 2.83^x	25.26 ± 1.88	35.20 ± 4.07^a	32.86 ± 2.11^{b,p}
GSH	8.05 ± 0.60	4.32 ± 0.42^z	7.41 ± 0.91^b	7.97 ± 0.92^b	8.67 ± 0.93^c
GPx	7.96 ± 0.44	5.33 ± 0.31^y	6.61 ± 0.27^b	7.51 ± 0.44^b	8.52 ± 0.47^{c,p}

[x]P<0.01, [y]P<0.001 & [z]P<0.0001 as compared to respective control; [a]P<0.05, [b]P<0.01 [c]P< 0.001 & [D]P<0.0001 as compared to respective diabetic group; [p]P<0.01 & [q]P< 0.001 as compared to respective drug treated group; [n]P<0.01 & [m]P<0.001 as compared to respective restricted diet treated group.

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Table 5: Effects of Diabecon (2 gm/kg BW) or restricted diet (RD; 50% of the control) either alone or both (Drug+RD) for 60 days on serum lipid profile. TC (total cholesterol; mg/dl), TG (triglyceride; mg/dl), LDL-C (loe density lipoprotein; mg/dl), VLDL-C (very density lipoprotein; mg/ dl), HDL-C (high density lipoprotein; mg/ dl) and TG /HDL ratio and AI (atherogenic index).

Parameters	Control	Diabetic	Diabetic + Drug	Diabetic + RD	Diabetic + Drug + RD
TC	140.94 ± 3.35	256.38 ± 10.61^z	194.01 ± 3.41^d	189.43 ± 6.58^d	147.65 ± 5.53^{d,p,m}
TG	66.21 ± 2.26	174.34 ± 5.69^z	109.49 ± 5.47d	100.63 ± 5.25^d	86.11 ± 3.85^{d,p,n}
LDL-C	80.28 ± 4.04	189.46 ± 10.66^z	132.97 ± 4.49^c	133.27 ± 6.48^d	87.84 ± 5.07^{d,q,m}
VLDL-C	13.24 ± 0.452	34.86 ± 1.14^z	21.89 ± 1.09^d	20.12 ± 1.05^d	17.22 ± 0.77^{d,p,n}
HDL-C	47.75 ± 3.11	32.04 ± 1.42^y	39.13 ± 3.06	36.03 ± 1.81	42.52 ± 2.99^b
TG/HDL	1.45 ± 0.147	5.49 ± 0.245^z	2.87 ± 0.216^d	2.83 ± 0.187^d	2.06 ± 0.108^{d,p,m}
AI	2.073 ± 0.278	7.09 ± 0.481^z	4.15 ± 0.443^c	4.34 ± 0.341^c	2.55 ± 0.278^{d,p,m}

^{x}P<0.01, ^{y}P<0.001 & ^{z}P< 0.0001 as compare to respective control; ^{a}P<0.05, ^{b}P<0.01 ^{c}P<0.001 & ^{d} P<0.0001 as compared to respective diabetic group; ^{p}P< 0.01 & ^{q}P< 0.001 as compared to respective drug treated group; ^{n}P<0.05 & ^{m}P<0.01 as compared to respective restricted diet treated group.
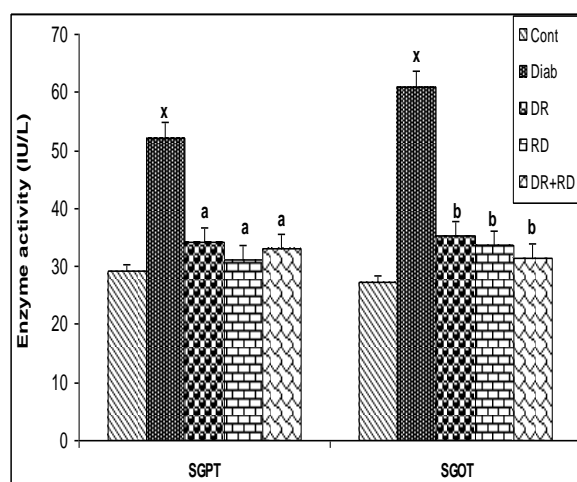
Figure 1: Effects of different treatments on serum glutamic oxaloacitic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) of experimental rats. Cont, normal control group; Diab, diabetic group; DR, diabetic + drug treated group; RD, diabetic group kept on 50% restricted diet; DR+RD, diabetic drug + 50% restricted diet treated group. ^{x}P<0.001, as compared to respective control values. ^{a}P<0.01 and ^{b}P< 0.001 as compared to the values of respective diabetic group.
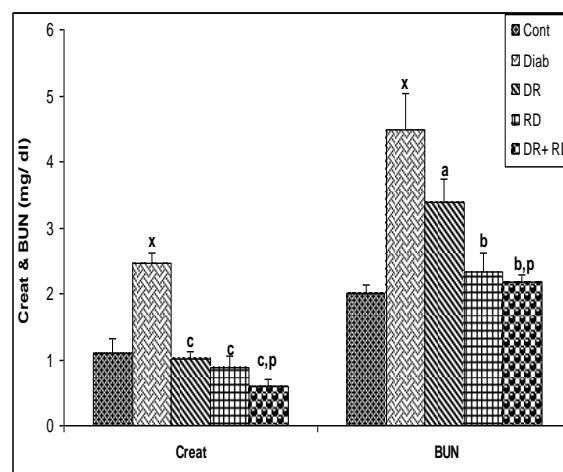
Figure 2: Effects of different treatments on serum creatinine (Creat) and blood urea nitrogen (BUN) of experimental rats. Cont, normal control group; Diab, diabetic group; DR, diabetic drug treated group; RD, diabetic group kept on 50% restricted diet; DR+RD, diabetic drug+50% restricted diet treated group. ^{x}P<0.001 as compared to respective control values. ^{a}P<0.05, ^{b}P< 0.01 & ^{c}P< 0.001 as compared to the values of respective diabetic group. ^{p}P< 0.01 as compared to respective values of only drug treated group.

DISCUSSION

Findings from the present investigation clearly indicate a better prevention of DM by the combined therapy of Diabecon and 50% restricted diet as compared to their individual therapy.

Other than reduction in serum glucose, a clear decrease in oxidative stress and increase in antioxidative status in all tested tissues also stated its beneficial effects on adolescent animals. The presently observed protective effects of RD corroborate with the earlier reports[5,35]. Anti-hyperglycaemic effect of Diabecon is also supported by the finding of Mitra[36]. However, this appears to be the first report revealing hepatoprotective, renoprotective and cardioprotective efficacy of Diabecon after chronic drug treatment.

The test drug decreased tissue LPO and enhanced the level of antioxidants such as SOD, CAT, GPx and GSH in the examined organs. This might be considered as sound argument in the induction of protective cell signalling pathways[16,17,34]. In addition, rats kept on RD showed a decrease in BW, physical activity and dullness in their behaviour for initial 3 weeks but later on, they recovered back to normalcy. Changes in BW were also supported by previous studies[10,37].

As, alloxan is an oxygenated pyrimidine derived beta-cytotoxin, that has been used as diabetogen for long[2,34], here also alloxan administration showed hyperglycaemia, hyperlipidemia[16,32,34] and marked increase in tissue LPO, LOOH and protein carbonyls with concomitant decrease in the activities of cellular antioxidants[6,7,32-34]. Decreased hepatic glycogen and increased activity of G6Pase are common features in diabetic rats[16,21].

Significant improvement was found in fasting glucose and liver glycogen content in animals treated with RD or ayurvedic formulation or both, than the diabetic group, again confirmed the anti-hyperglycaemic role of food restriction[11,35] and herbal drug therapy[15,17,34]. This study demonstrated the unknown synergistic effects of both therapies to prevent DM, oxidative stress and hyperlipidemia. Moreover, significant increased level of insulin in drug treated rats confirmed its insulinotrophic or β-cell protective activity[4]. In contrast to the drug treated rats, diet restricted animals showed comparatively less insulinotrophic effect, suggesting that it might control diabetes via different pathway(s)[17]. However, with respect to decrease in activity of G6Pase additive effects were seen in combined therapy group, which also affirmed their additive ameliorating effects.

Prolonged hyperglycaemia also induces non-specific glyco-oxidation and the formation of protein carbonyls, which promotes synthesis of advance oxidation end-products[25,38] along with non specific glycation of antioxidant defence machineries[6,7,16]. Since animals kept on single or combined therapy showed recovered activities of the cellular antioxidants, possibly the up-regulation of responsible genes is promoted by either therapy.

The liver is a major organ responsible for most of the metabolic functions[21,22]. Clinical diagnosis of oxidative stress and damage to the structural integrity of liver is commonly expressed by diminished activity of antioxidants, abnormal histological features along with higher activities of SGOT and SGPT enzymes[17,33,34].

In our study these parameters were significantly reversed in animals kept on different therapies. Histological alterations were also found to be normalized, suggesting that, the treatment with DT+RD may restore normal cell functions.

Numerous toxic effects on kidney and heart tissues have been reported in diabetic patients[16,20], also seen in our experimental diabetic rats[38]. However, improvement in these parameters were observed in animal given different therapies with decreased serum creatinine and BUN. These findings are also supported by earlier reports, where long term diet reduction or herbal treatment may induce expression of a number of genes that attenuate stress-sensitive signalling pathways such as *NF-κB, JNK/SAPK, CREB* and *p38 MAPK*[2,13].

Enhanced activities of antioxidative enzymes and GSH content, with a parallel decrease in oxidative stress markers (LPO, LOOH and PC) were in accordance with the earlier reports[10,19], suggesting that the observed beneficial effects could be the result of direct free radical scavenging of phytocompounds[4,6,14] and/or decrease in free radical generation[17,19,31].

In diabetic animals, hypercholesterolemia and hypertriglyceridemia might have resulted from increased rate of lipolysis and decreased gluconeogenesis in liver and adipose tissues[3,12].

Because, the level of TC, TG, LDL and HDL were found to be nearly normalized in drug, diet and drug + diet treated groups; we hypothesize that these therapies might have lipid lowering efficacy, as also suggested in other studies[4,20,37]. On the other hand, some polyphenolic and flavonoid compounds from *Allium sativum*, *Trigonella foenum graceum, Momordica charantia*, *Gymnema sylvestre* and *Azadirachta indica* showed activation of genes involved in lipid catabolism and their insulinotropic action promoting glycogenesis[4,19,22]. Increased insulin sensitivity and HDL level in RD+DT rats also corroborate with other reports[5,11].

Certainly, this is the first report with RD and polyherbal drug mediated improved health effects in non-obese diabetic rats.

Better normalization of almost all diabetes induced complications in combined therapy group exhibits the potential protective effects. In conclusion, the present findings suggest the possibilities that combined effects of diet restriction with herbal drug treatment might improve the general health of humans regardless of their body weight and age.

Possibly, this remedy proves to effective and safe for prediabeic or prone individuals with genetic history of DM, as both the therapies are chemical free natural and with least chances of side effects.

Of course, well controlled human studies are needed to ascertain the health benefits of RD + herbal therapy, particularly to slow down the progression of DM and related complications.

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